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INJURY INDUCED MOLTING IN *GALLERIA MELLONELLA* LARVAE

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Injury to larval or nymphal insects usually causes a delay in molting (O'Farrell and Stock, 1953; Pohley, 1959, 1960). When an appendage of a cockroach is amputated (O'Farrell, Stock and Morgan, 1956) or an imaginal wing disc is extirpated from lepidopteran larvae (*e.g.*, *Ephestia*, Pohley, 1960; *Galleria*, Madhavan and Schneiderman, 1969) there is a delay in the onset of the succeeding molt. This delay is particularly obvious when the injury is inflicted before preparations for the succeeding molt are initiated and appears to be proportional to the magnitude of injury. Based on these facts, injury induced delay of molting is attributed to the regenerating tissues (see Pohley, 1967; Bodenstein, 1959; and Madhavan and Schneiderman, 1969) which somehow inhibit the normal initiation of molt. As insect molt is controlled by brain and ecdysial glands (*cf.*, Novak, 1966), the injury may cause delay in molting by inhibiting either brain or prothoracic glands, but the actual mechanism of injury induced delay of molting is not clear. In contrast to this, injury to chilled, debrained, diapausing pupae of saturniid moth, *Hyalophora*, activities the prothoracic glands to secrete molting hormone and initiate adult development (McDaniel and Berry, 1967).

We examined the effect of injury on molting in last larval (7th) instar of *Galleria mellonella* and present evidence that early (less than 24 hr old) last instar larvae undergo an extra larval molt within 96 hours after injury, while mid instar larvae (4 day old) delay their molting. The results also suggest that injury induction of molting in early last instar larvae is mediated via the brain which appears to activate prothoracic glands and possibly the corpora allata. Furthermore, unlike during a normal larval molt, only a small percentage of the epidermal cells of larvae induced to undergo an extra larval molt, engage in DNA synthesis.

Materials and Methods

Galleria mellonella larvae are reared in the laboratory at 29° C, 70% R.H. on an artifical diet consisting of mixed cereal (Gerber Baby Foods), glycerine, honey and bee's wax in the proportion 12:1:1:0·5 by volume. In the strain of *Galleria* used in this study, freshly molted last instar larvae are colorless but become pigmented a few hours after molt. They can also be recognized by the size of the head capsule and body weight (Beck, 1970). Accurately timed larvae are separated from the culture and maintained in plastic petri dishes with food. These larvae normally pupate in about 8 to 9 days and in our laboratory do not normally undergo an additional larval molt unless injured.

Unless otherwise specified, the term larva refers to 7th (last) instar larvae which were used in all the experiments reported here. The terms early, mid

and late instar refer to less than 1, 4 and 6-day-old last instar larvae, respectively. Six-day-old larvae spin a coccoon on isolation but have not entered pharate pupal stage.

The larvae were routinely anaesthetized by placing them on crushed ice and the actual surgical maneuvers were also carried out while the larvae were on ice. Other modes of anesthesia applied in these studies were the use of CO_2 and etherization. The standard injury was to remove the right metathoracic wing disc. A mixture of phenylthiourea, streptomycin and penicillin (2:1:1) was placed in the wound to prevent infection and quinone formation and the wound was sealed by a blood clot formed during a 30 minute period on ice. In addition to the standard injury, transection of the ventral nerve cord between the 5th and 6th abdominal segments and integumental injuries were also used in some experiments. The integumental injury consisted of an operation to cut open the integument as if it were to remove the wing disc. The surgical procedures were similar

TABLE I

Effect of injury on molting in last instar larvae of Galleria mellonella

Age of larva in days	Treatment	Number of larvae	No. molted into larvae	No. of adults with regenerated wing discs
0 (0–6 hr)	Uninjured	15	0	—
0 (0–6 hr)	Wing disc removed	15	14*	13
1 (15–24 hr)	Uninjured	12	0	—
1 (15–24 hr)	Wing disc removed	25	24*	24
1 (15–24 hr)	Integumental injury	15	12	—
1 (15–24 hr)	Transection of ventral nerve cord	12	10**	—
4	Wing disc removed	18	0	15
6	Wing disc removed	12	0	0

* Some of these larvae underwent one more larval molt before pupation.

** Several of these larvae died after the larval molt. Four larvae, that survived, did not pupate in 3 weeks.

to those described by Schneiderman (1967; see also Madhavan and Schneiderman, 1969). Each of the experiments was repeated at least twice with essentially similar results. The data presented are those of a typical experiment.

Ecdysones used in this study were inokosterone and ecdysterone (Syn. β ecdysone) purchased from Rohto Pharmaceuticals, Osaka, Japan or obtained from Mann Research Corp., Rahway, New Jersey. Ecdysones were dissolved in 10% ethanolic insect ringer to a concentration of 5 μg/μl. Juvenile hormone (Roeller compound) was generously provided by Hoffmann La Roche, Inc., Nutley, New Jersey. This compound was a mixture of the *cis. trans* isomers and was about 30% as active as the pure hormone obtained from Prof. A. S. Meyer, of Case Western Reserve University, Cleveland. The hormone was dissolved in peanut oil. Tritiated thymidine (H^3TdR) was purchased from New England Nuclear Corp. The specific activity was 2.0 C/m*M* and was dissolved in sterile distilled water to a concentration of 1 μc/μl. Autoradiographic procedures were similar to those reported earlier (Krishnakumaran, Berry, Oberlander and Schneiderman, 1967).

Results

(1) *Injury induction of an extra larval molt*

In the first series of experiments, last instar larvae of known ages, varying from immediately after molt (0 day), 15–24 hrs (1 day), 4-day and 6-day-old, were anesthetized on ice, injured and examined for the induction of an extra larval molt or a delay in molting. The injury consisted of either removal of the right methathoracic wing disc, transection of ventral nerve cord, or an integumental injury. Uninjured controls pupated 8–9 days after the last larval molt. The results reported in Table I show that, irrespective of the type of injury, about 90% of the 0 day and 1 day old injured larvae molted into larvae within 4 days after injury. Occasionally these larvae underwent an additional larval molt before pupation. Injury to four-day-old larvae invariably caused a delay in the succeeding molt, which was a pupal molt, by about 6 to 8 days. Six-day-old last instar larvae also delayed pupation on injury but not to the same extent as the four day old injured larvae. The above results were obtained with larvae that were anesthetized on ice, however, when larvae were anesthetized under CO_2 or ether only 10 to 20% of the 0 and 1-day-old injured larvae underwent an extra larval molt. The larvae that did not undergo a molt showed a delay in pupation.

The injured larvae (in which metathoracic wing disc was extirpated) were allowed to complete adult development and the resulting adults were examined for the presence or absence of right metathoracic wing. The results show (Table I) that larvae from which wing discs were extirpated on day 1 or 4 of the stadium regenerated the wing (90%) while none of the larvae from which wing discs were removed on day 6 regenerated the wing. In one experiment, 10 larvae that completed the injury induced larval molt were dissected to determine whether they regenerated the wing disc during this molt. These larvae showed an accumulation of a tracheal mass at the site of injury but no regenerated wing disc.

(2) *Effect of juvenile hormone on injury induction of larval molt*

The titer of juvenile hormone in *Galleria* is high in the early part of the instar and is absent in older larvae (unpublished observations; *cf.*, Novak, 1966). Since this may account for the difference in the response of larvae to injury at different ages, we investigated the effect of juvenile hormone on injury induced larval molts. Injection of 2 or 4 μg of j.h. into 4 day old or 8 μg of j.h. into 6-day-old larvae did not induce a larval molt (such apparently high doses of juvenile hormone did not induce larval molts because the compound was a *cis. trans* mixture with only about 30% activity as the pure hormone *cf.*, Sehnal and Meyer, 1968). Following the application of these doses of j.h., the metathoracic wing disc was extirpated. Controls received an equal amount of peanut oil before injury. The results reported in Table II show that the presence of juvenile hormone at the time of injury did not influence the induction of molt, and regeneration of wing discs.

(3) *Effect of injury on juvenile hormone induced super larvae*

Early last instar larvae, unlike older larvae, respond to injury by initiating a larval molt. A larvae that molted once in response to injury undergoes an additional

molt if injured again soon after the molt. Is this ability to respond to injury limited to injury to natural 7th instar larvae only; or do 3 and 4-day-old last instar larvae induced to undergo an additional larval molt by injection of an adequate dose of juvenile hormone (Sehnal and Meyer, 1968) respond to injury by undergoing an additional larval molt. To determine this, several 3 to 4-day-old last instar larvae were injected with 10 μg/larva juvenile hormone: a dose adequate to cause perfect additional larval molts in 4 to 8 days. J.h. induced super larvae when left untreated, continued to feed, completed normal larval development and pupated 9 to 12 days later. Ten of these larvae were injured, by removal of a wing disc, on the day of molt while another ten were maintained as untreated controls. These larvae were examined to determine the time and nature of the succeeding molt. Both controls and experimental animals pupated in about 10 to 12 days without an additional larval molt. Furthermore, these larvae did not regenerate the wing disc before pupation. These results show that j.h. induced super larvae differ from normal last instar larvae in their response to injury.

(4) *Role of brain in induction of supernumerary molt*

Is the injury induction of larval molt in early 7th instar *Galleria* larvae the result of activation of brain or ecdysial glands? To determine this, 60 one day

TABLE II

Effect of juvenile hormone on injury induced larval molt

Age in days	Amount of j.h. injected (μg)	No. of larvae	No. undergoing additional larval molt	No. undergoing pupal molt	No. of adults regenerating wing discs
1	4	15	9*	9	9
	control	15	12*	12	12
4	2	15	0	11*	9
	4	15	0	9*	9
	control	15	0	10*	—
6	4	12	0	9**	0
	8	12	0	8**	0
	control	12	0	10	0

Controls received an equal volume of peanut oil.
* Remaining larvae died.
** The others died before pupation. Usually these delayed pupal molt by 7 to 10 days.

old larvae were injured and immediately after injury, 24, 48, or 72 hrs later, they were ligated behind the brain. Uninjured 1, 2, 3, and 4 day old larvae were ligated and served as controls. These preparations were maintained for 15 days and examined for deposition of a new cuticle. All 50 control larvae and all larvae ligated within 48 hr after injury failed to deposit a new cuticle. About 20% of larvae that were ligated 72 hr after injury deposited a new cuticle. These results show that the brain is essential for injury induction of a supernumerary molt. Furthermore, these data also suggest that injury does not induce the molt either by activating the prothoracic glands or by directly influencing the chitogenous epithelium.

Direct evidence for the role of the brain in the induction of an extra larval molt was obtained by implantation of brains from injured early larvae into older injured larvae. Several one day old last instar larvae were injured. One or two days later, their brains were carefully dissected out and cleaned of all attached glands and nerves and implanted into 4 and 6-day-old larvae. Routinely two brains were implanted into each 6-day-old larva, while 4-day-old larvae received only one brain. In another experiment the head capsule of an injured larva containing the brain, its associated glands, and subesophageal ganglion, was implanted into 4-day-old larvae. Controls received brains or head capsules from uninjured 2 or 3-day-old larvae. These were examined for the induction of molt and if they deposited a cuticle the nature of molt was also determined. There were no differences between the larvae that received a brain alone and those that received the head capsule with brain and the associated neuroendocrine glands.

Results are reported in Table III, and show that the brain from uninjured 2 to 3-day-old larvae (unlike those of injured larvae) did not induce a precocious molt, confirming the earlier observations of Beck (1970). In one limited experiment (with 4 larvae) 3 brains from uninjured early larvae were implanted into each larva, but even these failed to induce a prompt molt whether larval or pupal. However, over 70% of the 4 day old larvae that received a brain from injured

TABLE III

Effect of implantation of injured early larval brain on molting in 4 and 6 day old larvae

Stage and treatment		No. of brains implanted	No. of recipient larvae	No. undergoing a prompt molt
Donor	Host			
3-day-old normal larva	4-day-old intact larva	1	12	0
2–3-day-old injured larva	4-day-old intact larva	1	25	17*
3-day-old normal larva	6-day-old intact larva	2	10	0
2–3-day-old injured larva	6-day-old intact larva	2	22	4*
3-day-old normal larva	4-day-old larva ligated behind head	2	12	1**
2–3-day old injured larva	4-day-old larva ligated behind head	2	12	7**
—	4-day-old larva ligated behind head	0	12	0

* The additional molt was induced in 4 to 6 days and was usually larval. One larva molted into a pupa.

** This molt was pupal except in two cases and occurred between 11 and 16 days after implantation of brains.

larvae underwent an extra larval molt within 4 to 6 days. Such super larvae subsequently pupated and developed into apparently normal adults. Although the implanted brain usually induced an extra larval molt, occasionally a larva pupated promptly in response to the implanted brain. A small number of 6-day-old larvae also molted into larvae 7 days after implantation of 2 activated brains. One of these larvae had a larval body with everted imaginal wing discs and pupal tanning in the middorsal line of the thoracic and anterior abdominal segments, and was capable of larval-type movements. Similar larval-pupal intermediates were obtained by injection of j.h. in 6-day-old *Galleria* larvae by Sehnal and Meyer (1968; Krishnakumaran, unpublished observations).

TABLE IV

Pattern of DNA synthesis in various tissues during normal and injury induced larval molts in Galleria

Tissue age in days	6th Instar	7th Instar							
	Normal larval-larval molt 1–5*	Uninjured larvae			Injury induced molt				
		1	2	3	1	2	3	4	1–4**
Epidermis	++++	0	0	+	0	0	+	+	+
Trachea	++	0	0	−	0	0	+	+	+
Midgut	+++	+	+	+	+	+	+	+	+
Fat body	++++	+	+	+	+	+	+	+	+
Muscle	+++++	+	+	+	+	+	+	+	+
Hemocytes	++	+	+	+	+	+	+	+	+
Malpighian tubules	+++++	+	+	+	+	+	+	+	+

+ Incorporated H^3TdR. Each + represents up to 15% of cells examined.
0 No incorporation of H^3TdR. − Data not available.
Larvae were injected with 3 μc/larva H^3TdR and sacrificed 24 hr later.
Four larvae were examined on each day.
* Larvae were injected with 1 μc/larva H^3TdR on each of the five days of the 6th stadium.
** Larvae were injected with 3 μc/larva H^3TdR at the time of and, 48 and 72 hr after injury.

Further evidence to show that brains from early 7th instar larvae are capable of inducing a molt was obtained by implantation of the brains into ligated larval abdomens. Four day old *Galleria* larvae when ligated behind the head capsule so as to deprive them of a source of brain hormone, survive for several weeks but do not molt. Twelve of these ligated larval abdomens received two brains each from injured early larvae. Controls either received brains from 2 to 3-day-old uninjured larvae or were untreated. Between 11 and 16 days after implantation of the brains, 56% of the larvae that received the injured larval brain and 8% of the controls molted (Table III) while none of the untreated controls molted even after 4 weeks. The molt induced by the implanted brain was usually a pupal molt although, in one experiment, two larval abdomens deposited a distinctly larval cuticle with well developed prolegs, larval bristles and uneverted wing and leg imaginal discs. Possibly in these two preparations allata were implanted along with the brain.

These results show that brains from injured, unlike those of uninjured early 7th stadium *Galleria* larvae are capable of activating the ecdysial glands.

(5) *Injury induced delay in molting*

Mid (4 day) and late (6 day) last instar larvae when injured, delay subsequent pupation. This delay in molt could be the result of inability of (a) the chitogenous epithelia to respond to ecdysones, (b) the ecdysial glands to brains hormones, or (c) the failure of the brain to activate the ecdysial glands. The fact that active brains (from injured early larvae) initiated a molt in injured 4-day-old larvae suggested that the ecdysial glands are not refractile. Moreover, these results also show that the epidermal cells of injured mid larvae are not unresponsive to

ecdysones. This conclusion was further supported by injection of exogenous ecdysones. Twelve injured 4 day old larvae were injected with 10 μg/larva ecdysterone soon after injury, and control larvae received Ringers' fluid. While all controls pupated in 8 to 10 days, all ecdysterone treated larvae deposited a new cuticle within 72 hours after injection, showing that the epidermal cells of these injured larvae can respond to ecdysone. Further, it is interesting to note that ecdysterone treated larvae secreted a larval-pupal intermediate type of cuticle. Thus, it was concluded that the injury induced delay in these larvae is not due to inability of chitogenous epithelium to respond to ecdysone and that the delay may be mediated via the brain.

The next question we asked was, whether the inactivity of the brain in injured 4-day-old larvae was the result of absence of stimulus or the presence of a molt inhibitory agent. To determine this, two brains from injured 4-day-old larvae were implanted into each injured 1-day-old larva. If a molt inhibitory factor was present in these brains, it may be expected to suppress or delay the injury induced molt in the host larvae. All ten larvae that received these brains, molted about the same time as the controls, suggesting that injury induced delay of molting in mid larvae was not the result of the presence of a molt inhibitory hormone but may be due to the absence of the normal stimulus that activates the brain.

(6) *DNA synthesis during normal and injury induced larval molts*

In the course of normal larval molt, epidermal cells in a variety of insects engage in extensive DNA synthesis (Bowers and Williams, 1964; Krishnakumaran *et al.*, 1967). What are the patterns of DNA synthesis in the various tissues during a normal and injury induced molts in *Galleria*? We examined this by determining, autoradiographically, the incorporation of H^3TdR in 6th stadium larvae (penultimate larval instar) and during an injury induced molt in 7th instar larvae.

Six larvae were isolated soon after their molt into 6th stadium. These received 1 μc/larva H^3TdR on each of 5 days of this stadium, and were killed soon after molting into 7th stadium larvae. Autoradiographic analysis of the pattern of incorporation of H^3TdR in different tissues of these larvae showed that about 70% of the epidermal cells, 80% of the muscle cells and malpighian tubule cells and 60% of the fat body cells incorporated H^3TdR into DNA of their nuclei (see Table IV). These results are comparable to the observations made on larval *Cynthia ricini* (Krishnakumaran *et al.*, 1967).

The pattern of DNA synthesis during an injury induced larval molt was examined next. For this purpose 16 newly molted 7th stadium larvae were injured. Immediately after injury (0 day), 1, 2, or 3 days after injury, these larvae were injected with 3 μc/larva H^3TdR. These larvae were sacrificed 24 hr after injection of isotope. Another batch of 4 injured larvae was injected with 3 μc/larva H^3TdR immediately after, 48 and 72 hr after injury. These larvae were killed after the subsequent molt and autoradiographs of tissues away from the site of injury were prepared. Uninjured larvae served as "controls." Autoradiographic data regarding the numbers of nuclei that incorporated thymidine are presented in Table IV and indicate that during the first 48 hr of the last instar there was no incorporation of H^3TdR in the epidermal cells and even in the other tissues, DNA synthesis was not extensive. In larvae that received isotope between 48 and 72 hr 10–15%

of epidermal cells engaged in DNA synthesis. During the first 48 hr of an injury induced molt only about 5% of the cells in muscle, fat body, malpighian tubules and other tissues incorporated tritiated thymidine. As in uninjured controls, no labelled epidermal cells were observed in larvae during the first 48 hours after injury. Even in larvae that received the isotope 48 or 72 hours after injury only 4 to 5% of the epidermal cells, fat body cells, hemocytes, muscle cells and malpighian tubule cells engage in DNA synthesis. Thus three day old uninjured "controls" show a higher rate of incorporation of H^3TdR than the corresponding injured larvae. Even in larvae that were exposed to isotope throughout the entire period of injury induced molt, only about 10% of the fat body, muscle, epidermal, malpighian tubule and pericardial cells incorporated label. Thus, unlike in the normal larval-larval molt (during a penultimate larval stadium), DNA synthesis in cells of larvae during an injury induced molt was much more restricted.

Discussion

Role of the brain in the injury induction of molt

The results reported in the present account clearly show that injury to last instar larvae within 24 hr after molt, leads to an additional larval molt. However, older larvae do not respond in the same manner. The results also show that the brain is involved in injury induced molting in *Galleria* larvae. The fact that larvae ligated behind the brain as late as 48 hr after injury fail to molt, and that brains from injured early last instar larvae induce molting in intact mid and late last instar larvae as well as larvae ligated behind the head capsule, support this conclusion. As brains from uninjured 7th stadium larvae of corresponding chronological age do not activate ecdysial glands and induce a molt (a finding that confirms the earlier observations of Beck (1970) in these larvae), and the fact that the brain from injured larva is capable of initiating a molt suggests that injury somehow activates the brain. The mode of activation of the brain in lepidopteran larvae is obscure, but may involve the nervous system as was suggested by Edwards (1966). This conclusion is supported by the experiments using bracon venom (a potent inhibitor of neural activity), (Edwards and Sernka, 1969), physical restraint (Edwards, 1966) and nerve transection (Sehnal and Edwards, 1969) all of which inhibited normal metamorphosis of *Galleria* larvae. The present observations on the nature of response to injury under different anaesthetic agents also support such a conclusion. While larvae anesthetized on ice and injured underwent a prompt larval molt, larvae anesthetized under CO_2 or ether failed to respond in a similar way. These three anesthetics are known to affect nerve conduction in invertebrates differently (Caldwell, 1958; Hafemann, 1969). Carbon dioxide anesthesia results in an irreversible loss of ability to transmit nerve impulses, while cold does not cause such an irreversible change in invertebrate nerves (Hafemann, 1969). Preliminary studies on the isolated ventral nerve cord from *Galleria* 7th stadium larvae showed similar differences between CO_2 and cold anesthesia. Moreover, etherization and nitrous oxide also cause irreversible damage to the nerves (Hafemann and Krishnakumaran, unpublished observations). (The fact that Pohley (1960) and Madhavan and Schneidermann (1969) did not observe in *Ephestia* and *Galleria* larvae any injury

induced larval molts may be attributed to two factors. First they used older larvae and secondly they used ether for anesthetizing the larvae.) Thus, it is suggested that injury to early last instar larvae somehow mimics the normal stimulus that activates the brain and that this stimulus may be neural. Consequent to this, the early last stadium larvae undergoes molt on injury.

Although the early 7th stadium larva responds to injury by a prompt molt, injury to 4-day-old larvae causes a delay in the subsequent molt. The fact that injured larvae respond to exogenous ecdysones, by depositing a cuticle, suggests that the epidermal cells are not inhibited by the regenerating tissues. This finding confirms similar observations by Madhavan and Schneiderman (1969). In addition, the fact that implantation of an activated brain into such injured larvae induced a prompt molt, indicates that the prothoracic glands are not inhibited by the injury or the regenerating tissues. All these suggest that the delaying effect of injury to mid last instar larvae must also be mediated via the brain. The fact that implantation of brains from injured 4-day-old larvae into injured early last instar larvae did not cause a delay in initiation of molting suggests that the injury induced delay does not involve a molt inhibiting hormone but is possibly the result of the absence of the stimulus to activate the brain. However, it is not clear why the early and mid last instar larvae behave differently in response to injury.

Activation of allata

Injury to early last instar larvae caused an additional larval molt in about 4 days, which may be the result of the presence of juvenile hormone in the larvae at the time of initiation of this molt. In 24 and 48-hour-old last instar *Galleria* larvae extractable juvenile hormone titers are present and hence injury induction of larval molting is not surprising. However, implantation of brains from injured early larvae into 4 or even 6-day-old larvae (at which stages there is no extractable juvenile hormone) also induced larval molts. There are several indications that 4 to 6-day-old larval epidermis will deposit larval cuticle only if there is sufficiently high titer of juvenile hormones. For example on injection of beta ecdysone in 4-day-old larvae they deposit a larval-pupal intermediate type cuticle while 6-day-old larvae deposit a pupal cuticle suggesting that the epidermal cells at this stage secrete a pupal cuticle if forced to deposit a cuticle without any delay (*cf.*, Krishnakumaran, Granger and Schneiderman, unpublished observations). Sehnal and Meyer (1968) reported that 4 to 6-day-old 7th instar larvae deposit a larval cuticle only on application of large doses of juvenile hormone suggesting that normally 4 to 6-day-old 7th stadium *Galleria* larvae are committed for synthesis of pupal cuticle and that only in the presence of sufficient quantity of juvenile hormone do these larvae reprogram to deposit a larval cuticle.

How does a brain from an injured early 7th instar larva induce a larval molt in 4 and 6-day-old larvae? A likely explanation is that the implanted brain activated allata to secret juvenile hormone. The fact that implantation of a brain, devoid of all attached connectives and glands, induced larval molts, suggests that the implanted brain can activate the host corpora allata as well as the ecdysial glands. This view is further supported by the observation that 4-day-old larvae ligated behind the head usually pupate in response to implantation of an injury activated brain. (Ligation behind the head capsule removes brain and

also the allata but retains the ecdysial glands.) From these observations it is concluded that injury activated brains possess not only prothoracotropic but also allatotropic activity. Injury activation of allata is not uncommon in insects. In *Leucophaea maderae* Luscher and Engelmann (1960) reported that injury caused a continued activity of allata and supernumerary molts. Similarly amputation of antennae in *Periplaneta americana* early in a stadium reduced the rate of metamorphosis (Pohley, 1962). Whether the allatal activity in these two insects is regulated by a blood borne factor or by a nerve stimulus is not certain. The fact that in *Leucophaea*, transection of allatal nerves did not affect the induction of supernumerary nymphal molts suggests that the injury induced allatal activity does not require neural stimuli. In the earwig, *Anisolabis*, Ozeki (1962) reported injury stimulation of allata. However, in this insect nerve stimuli were shown to be necessary for this activation. The allatotropic factor in *Galleria* larvae may be a blood borne factor and is presumably a neurosecretory product as in some orthopterans (*Schistocerca*, Strong, 1965; *Locusta*, Girardie, 1966).

DNA synthesis and molting

The results reported here show that larval epidermal cells during the course of an injury induced larval molt are not actively engaged in DNA synthesis unlike during a normal larval-larval molt (penultimate larval instar of *Galleria* and 4th instar larvae of *Cynthia ricini*, Kirshnakumaran *et al.*, 1967). The number of cells that were engaged in DNA synthesis during an injury induced molt appears comparable to the number that normally incorporate H^3TdR during the first two days after the last larval molt. However, in 3-day-old last instar larvae, 10 to 15% of the epidermal cells engage in DNA synthesis which is more than in the injury induced molting larvae of corresponding chronological age. Thus it is clear that DNA synthesis during injury induced larval molt is not as extensive as in the penultimate instar larvae undergoing a normal larval molt, and that very few epidermal cells in these larvae replicate their DNA prior to molting. This condition reminds one of the pattern of DNA synthesis in the epidermal cells during adult-adult molts in *Cynthia* moths grafted to pupae (Krishnakumaran *et al.*, 1967), and in the ecdysterone injected *Cynthia ricini* larvae and *Tenebrio* pupae (Krishnakumaran, Granger and Schneiderman, unpublished observations).

There are several possible explanations for the relatively low level of DNA synthesis in epidermal cells of injury induced molting larvae. First, contrary to earlier suggestions, DNA synthesis normally noticed in the epidermal cells of larval insects in preparation to molting is unrelated to the presence of ecdysones (Kirshnakumaran, 1962; Bowers and Williams, 1964; Krishnakumaran *et al.*, 1967). Secondly, injury induced molt differs from the normal larval molts in the absence of an increase in size of the larva. Consequent to the lack of increase in size, there is no need for an increased number of cells and this resulted in a molt in the absence of extensive DNA synthesis in the larval cells (*cf.*, Wigglesworth, 1964). Thirdly, as injury induced the molt, even before the synthetic processes for the preceding molt were completed (*e.g.* deposition of the inner layers of the endocuticle and secretion of wax and cement layers of the epicuticle; processes which are under hormonal control, *cf.*, Locke, 1970), the levels of ecdysone may be continuously high throughout the entire injury induced molt cycle. The

low level of DNA synthesis in larval cells during an injury induced molt in *Galleria* larvae may be the result of the presence of high titers of beta ecdysone which is known to inhibit DNA synthesis and promote cuticle deposition (Oberlander, 1969, 1972).

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Summary

1. Injury to less than 1-day-old last instar larvae of *Galleria* induced an additional larval molt.

2. 4 and 6-day-old larvae on injury delay their succeeding pupal molt.

3. Injury induction of an additional molt as well as the delay of molting appear to be mediated via the brain.

4. Brains from injured early last instar larvae, unlike those of the uninjured larvae, appear to possess prothoracotropic and allatotropic activities.

5. During an injury induced molt the epidermal cells do not engage in DNA synthesis to the same extent as in the normal larval molts.

6. The relevance of these results in the analysis of control of molting and DNA synthesis is discussed.

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